RESTORE ACT CENTER OF EXCELLENCE FOR LOUISIANA FINAL TECHNICAL REPORT

Due within 30 days of the close of the award

Project Title: Patch-scale Effects of Acute Saltwater Intrusion on Carbon Fluxes in a Simulated Coastal Freshwater Marsh Environment

Principal Investigator:	Jorge A. Villa
Principal Investigator Institution:	University of Louisiana at Lafayette
Co-Principal Investigator:	Diana Taj
Co-Principal Investigator Institution:	University of Louisiana at Lafayette

A. TECHNICAL ACTIVITIES

1. Research Summary

1.1 Methods:

Study Site: This study was conducted in the Visser's Experimental Wetland Complex located at Cade Farm in St. Martinville, Louisiana. This experimental complex has eight constructed ecosystem-scale wetland units (50 x 20 m) used for ecological research in fresh and brackish water conditions. Four of the eight wetlands intended for brackish wetland research were constructed with underlying industrial-grade High-Density Polyethylene (HDPE) plastic liners that prevent potential pollution of the underlying aquifer system with the salt water used for experimentation (Fig. 1A). In 2013, after construction, native vegetation species characteristic of freshwater and brackish wetlands in Louisiana were transplanted from the Rockefeller Refuge and the White Lake Preserves, including the two common upper estuary plants that are featured in this study, *Typha domingensis* and *Panicum hemitomon*.

Recreating saltwater intrusion events: Using the four lined wetlands in the complex, we recreated saltwater intrusion (SWI) events of 2 ppt salinities with durations (i.e., treatments) of one, three, and five days. Each of these three events took place in different wetlands, with the fourth wetland as a control with no salinity manipulation. It remained lower than 0.5 ppt throughout the study but had similar water level fluctuations to that of the treatments. To create the SWI, we pumped freshwater into the wetland to raise the water level and increased the salinity with saline brines created with Instant Ocean™. The target for water level increase was 0.3 to 0.5 m, and for salinity increase, it was 2 ppt. To initiate an SWI event, we progressively added the brine and waited 24 hours after the last addition for stabilization before sampling. To end the event, we pumped out saline water and pumped in new freshwater until the salinity returned to freshwater conditions (i.e., < 0.5 ppt) and the water level dropped to previous levels. Before sampling, we allowed a 24 to 48-hour buffer stabilization time as part of the simulated SWI retreat. Experimentation started on 8/18/2022 and lasted until 10/24/2022.

Sampling greenhouse gas fluxes: For each treatment and control, we measured carbon dioxide (CO_2) and methane (CH_4) fluxes before, during, and after the recreated SWI events. We considered flux measurements during and after in one group for analysis. The measurements were conducted in distinguishable patches of *T. domingensis* and *P. hemitomon*, on 15 m^2 rectangular plots (2 plots per patch in each wetland). We installed two 30.5 × 30.5 cm (9.3×10^{-2} m^2) custom-made PVC collars at each plot (Fig. 1 B,C). For sampling fluxes from the wetland surface (i.e., soil-water surface and vegetation), we fitted the collars with a transparent chamber top connected to a portable greenhouse gas analyzer (Picarro GasScouter G4301™), creating a closed loop or steady-state flux chamber (Fig 1E). The tops had different top volumes to better fit the species biomass difference, 14.2×10^{-2} m^3 and 5.7^{-2} m^3 for *T. domingensis* and *P. hemitomon*, respectively. We also deployed a circular floating chamber with smaller dimensions (367.8 cm^2 surface area, 4.7 cm^3 volume) inside the collar immediately before each wetland surface chamber deployment to measure fluxes only from the soil-water surface (i.e., no vegetation) (Fig. 1F). Each chamber deployment in a wetland or soil-water surface lasted 2 minutes. The data series of CO_2 and CH_4 concentrations over time from the chamber runs were processed following the procedure described by Villa et al. (2021) to distinguish the diffusive and ebullitive contribution to the measured flux.

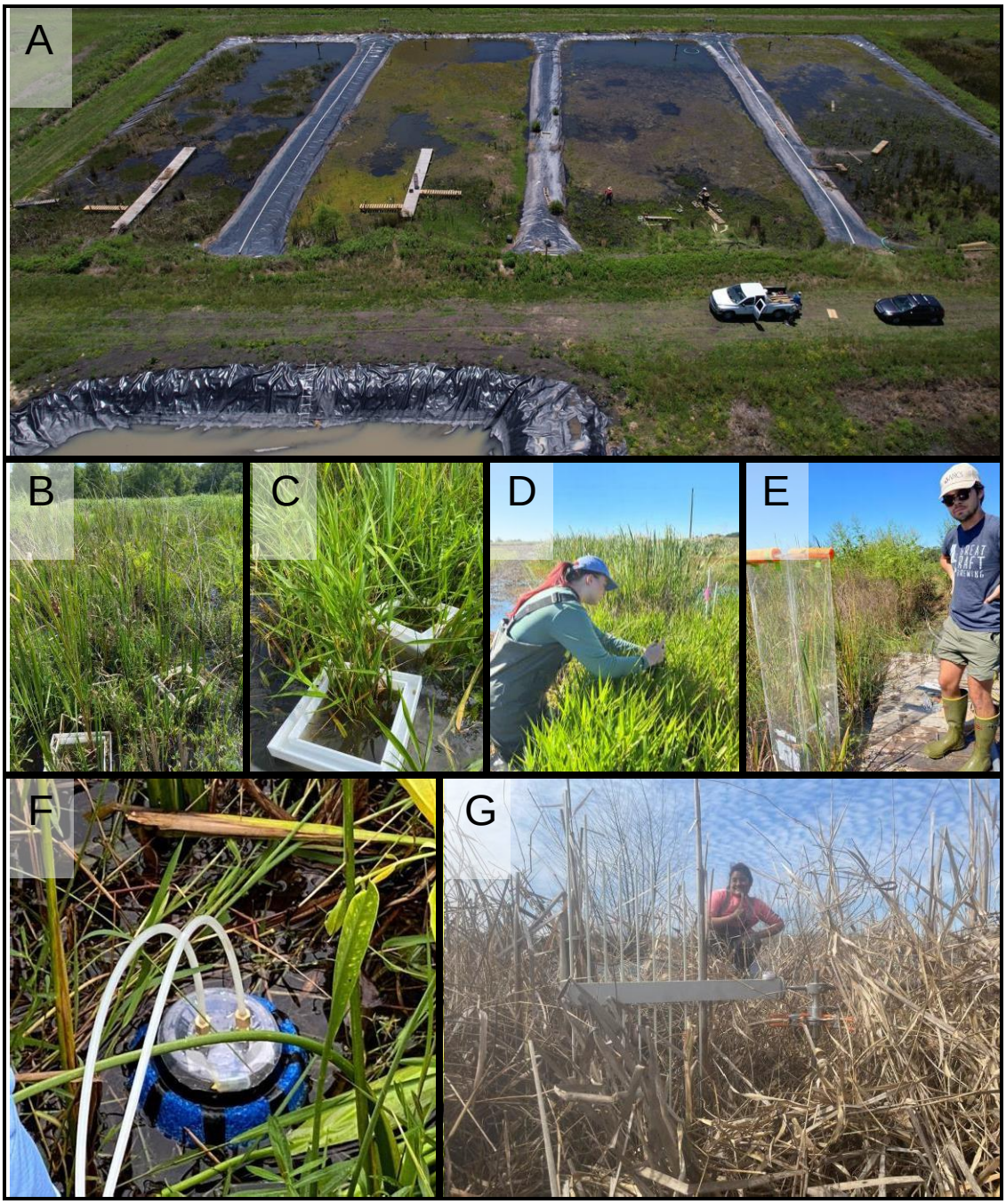


Figure 1. Experimental Wetland Complex (A), sampling collars in Typha patch (B), sampling collars in Panicum patch (C), measurements of spectral indexes (D), steady-state sampling chamber (E), the floating chamber for measurement of fluxes from the water column (F), and SET measurements (G).

Complementary measurements: Concomitantly to fluxes, we measured leaf absorbance and reflectance using a handheld leaf spectrometer to determine spectral indices proxies of plant health and appearance (i.e., NDVI, PSI, PSRI) (Fig. 1F). We also evaluated potential elevation changes resulting from simulated SWI events at each plot with surface elevation tables (SET) that were measured twice on 2/25/2022 and 2/25/2023 (Fig 1G). Salinity and water levels were measured continuously before, during, and after the SWI events with a Campbell Scientific CS547A-L sensor, a handheld YSI Model 85, and a Campbell

Scientific CS451 (Stainless-Steel Pressure Transducer), respectively. Photosynthetic available radiation (PAR) was recorded with LI-COR LI-190R Quantum sensor.

1.2 Main findings:

CO_2 flux observations in all three simulated SWI intrusion events and two different patches pooled together were positively correlated with PAR and PRI and negatively correlated with water level (see vectors in the same and opposite direction, respectively in Fig. 2A). In turn, CH_4 fluxes were also positively correlated with PRI and negatively correlated with PSRI (Fig. 2B). There is no apparent grouping of the observations by type of treatment (i.e., control or different durations of SWI) for CO_2 or CH_4 fluxes.

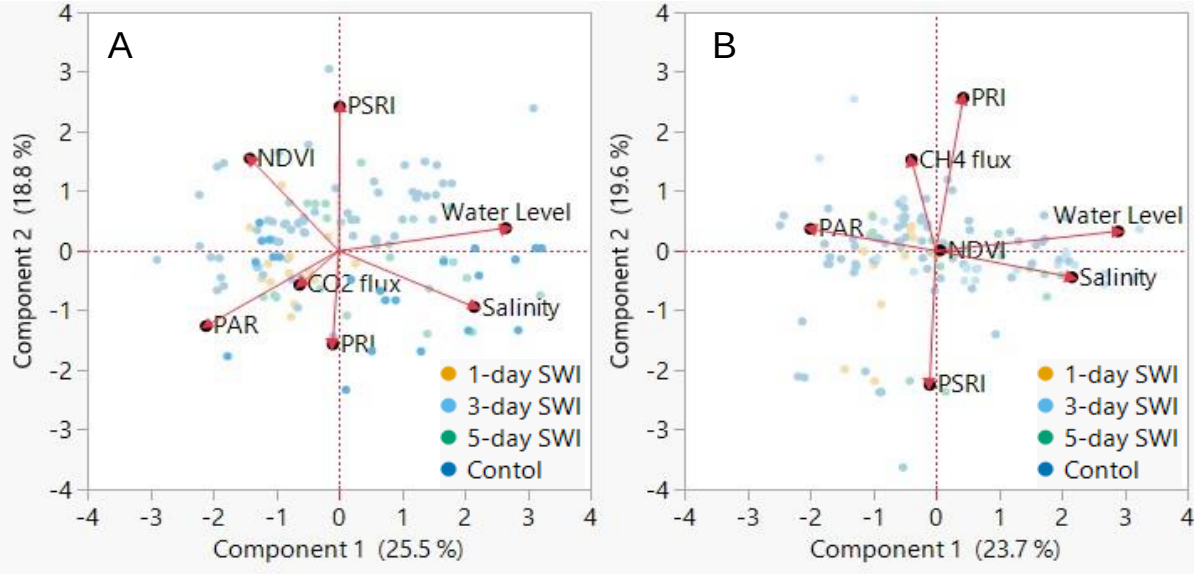


Figure 2 *. Principal component analysis biplots showing the association between the main variables considered in the study presented as vectors (red arrows). Panels show the two main principal components for (A) CO_2 and (B) CH_4 fluxes, accounting for 44.5% and 43.5% of the variability of the data, respectively. The variables considered in the analysis are salinity, water level, photochemical reflectance index (PRI), plant senescence reflectance index (PSRI), Normalized Difference Vegetation Index (NDVI), and Photosynthetically Active Radiation (PAR).*

Similar angles between vectors indicate vector association, while angles close to right angles indicate non-association between vectors.

CO_2 and CH_4 fluxes in the patches responded differently to the recreated SWI events (Fig. 3 and 4). Overall, we noticed less variability in CO_2 and CH_4 fluxes after the SWI events. In the *Typha* patches, we did not find significant effects of time (Before/After) and treatment (Control/Treatment), or their combination, in CO_2 fluxes, indicating that patches dominated by this species were able to withstand SWI events like the ones we simulated without undergoing major changes in CO_2 uptake rates by plants and respiration from the plants and soil, at least in the short term we evaluated. In contrast, we found significant interactions between time and treatment at *Panicum* patch, but only in the SWI of 3-day duration ($p < 0.05$, Fig. 4B). CO_2 fluxes decreased, indicating an increase in CO_2 uptake by the plants (photosynthesis minus respiration) and reduction of respiration of heterotrophic microorganisms along the soil-water column continuum.

The effect of the interaction between time treatment was not strong enough to create a significant difference in CH_4 fluxes within either vegetation patch. Only in the *Panicum* patch, we observed an effect of time after the 5-day SWI ($p < 0.05$) (Fig. 4F). Longer durations of SWI lead to fewer CH_4 fluxes, but when SWI were short-lived (i.e., < 5 days), the fluxes were not immediately affected.

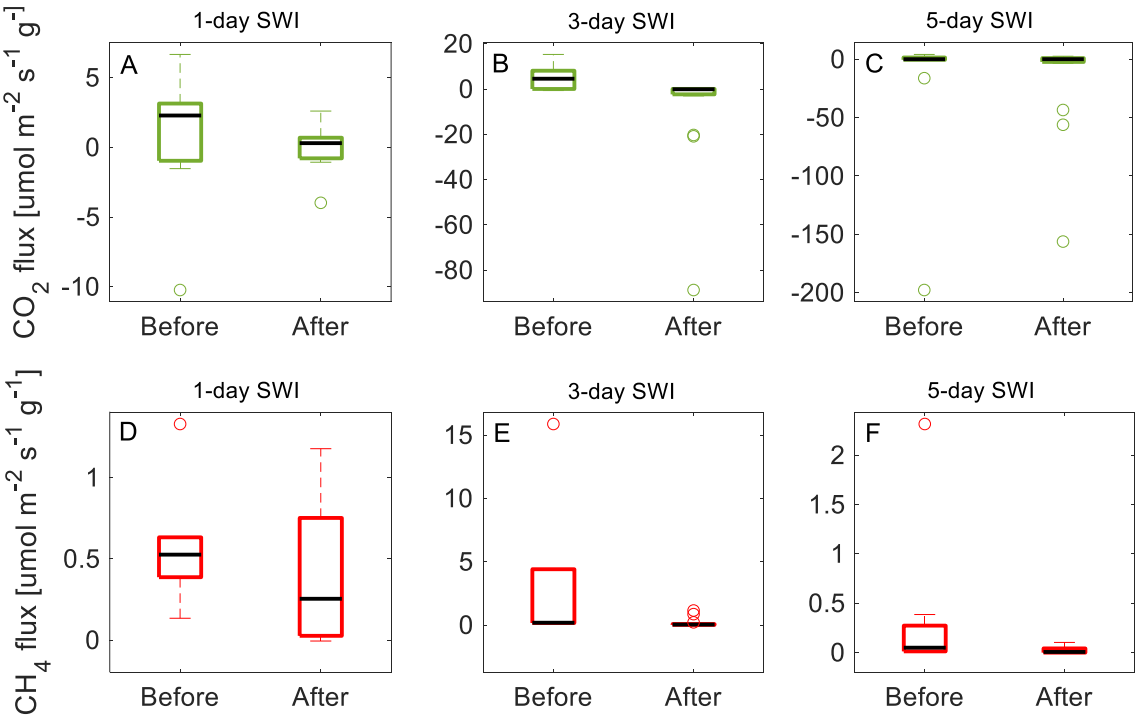


Figure 3. *Mean ± SE CO_2 (A,B, and C) and CH_4 (D, E, and F) fluxes measured in Typha patches during recreated SWI events of 1-day (A, D), 3-day (B, E) and 5-day (C, F) durations respectively. The effects of time (Before/After), treatment (Control/Treatment), and their interaction (time × treatment) on CO_2 and CH_4 fluxes were evaluated for a Before-After-Comparison- Impact experimental design using a two-way ANOVA.*

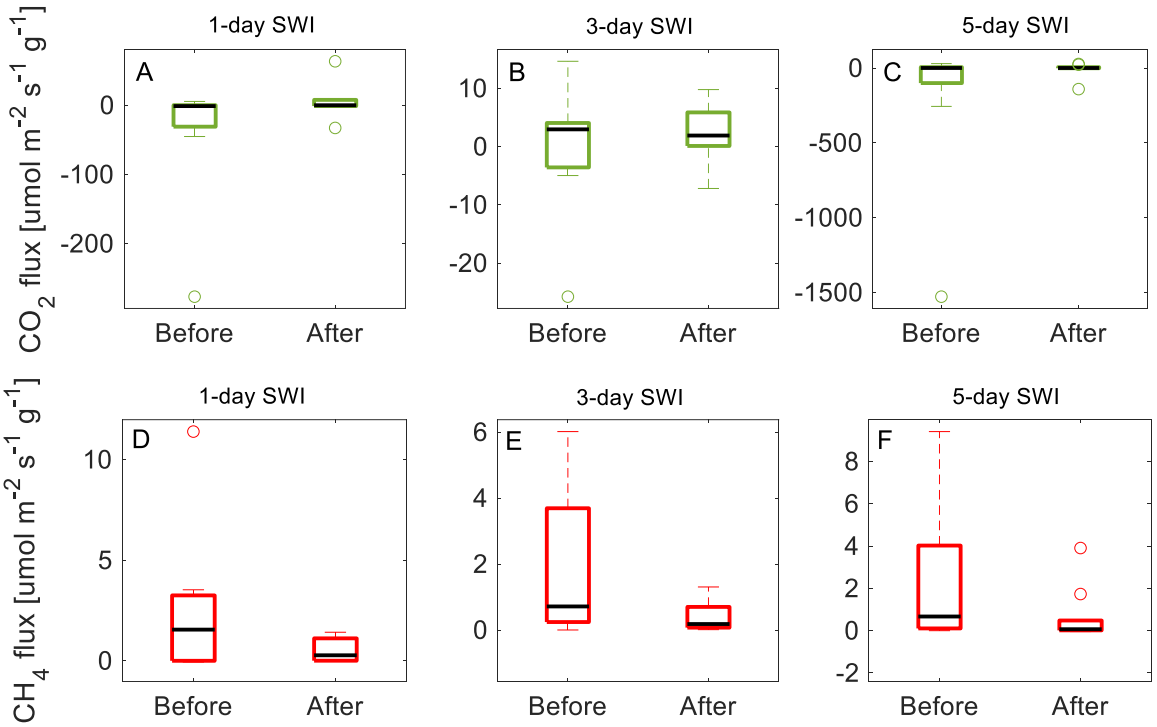


Figure 4*. Mean ± SE CO_2 (A,B, and C) and CH_4 (D, E, and F) fluxes measured in Panicum patches during recreated SWI events of 1-day (A, D), 3-day (B, E) and 5-day (C, F) durations respectively. The effects of time (Before/After), treatment (Control/Treatment), and their interaction (time × treatment) on CO_2 and CH_4 fluxes were evaluated for a Before-After-Comparison- Impact experimental design using a two-way ANOVA.*

We found differing changes in surface elevation between the two patches and the duration of SWI. The patches in the control and 5-day duration wetlands showed negative changes, indicating elevation loss (Fig. 5 A, D). In turn, the elevation changes in *Typha* patches exposed to 1- and 3-day SWI durations, and in the *Panicum* patches, only in the 3-day duration SWI gained elevation (Fig. 5 B, C). However, we don't think that these elevation changes, measured over one year, provide a robust evaluation of the potential effects of SWI, especially considering that experimentation ended in October of 2022 and there was a very short time (~ 3 months) for the system to register accurate changes in organic matter accumulation, the primary source of accretion in these experimental systems. However, we anticipate that these measurements will serve as a baseline in continuing experimentation (beyond this project) with SWI on the site.

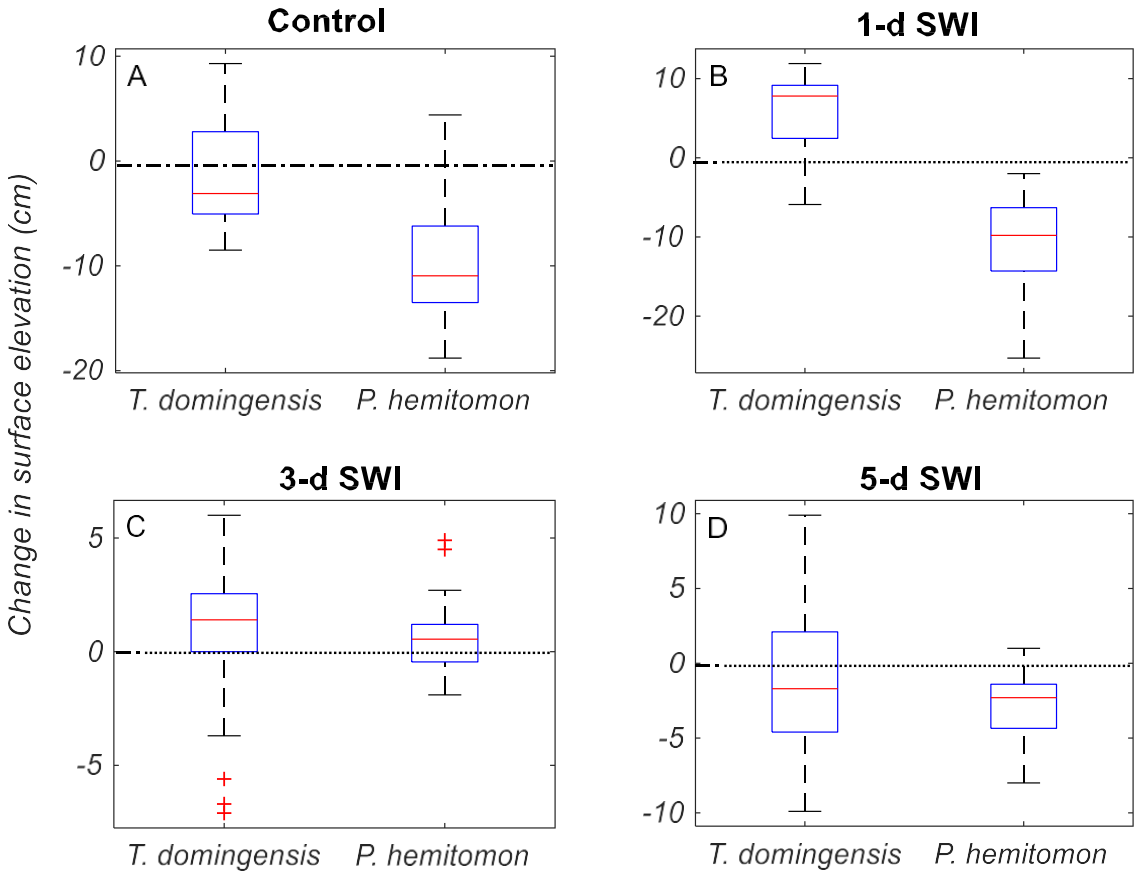


Figure 5. *Change in surface elevation between 2/25/2022 and 2/25/2023 measured with a surface elevation table (SET). The experimentation was completed in October of 2022.*

References:

Villa, J.A., Ju, Y., Yazbeck, T., Waldo, S., Wrighton, K.C., Bohrer, G., 2021. Ebullition dominates methane fluxes from the water surface across different ecohydrological patches in a temperate freshwater marsh at the end of the growing season. Science of The Total Environment 767, 144498. https://doi.org/10.1016/j.scitotenv.2020.144498

1.3 Application of research to implementation of Coastal Master Plan:

- Management of expectations in designing and implementing carbon sequestration projects in freshwater wetlands.
- Inform the vegetation subroutine in the morphology model of the Integrated Compartment Model (ICM), specifically in accounting for acute SWI and its effect on organic matter accumulation in future scenario projections.

B. DELIVERABLES

*Note – please submit all PDFs of reports, papers, and presentations with the final report **in the portal (LA-COE Apply)**. Thank you!*

1. **Deliverables on proposed goals and objectives.** If a goal or activity is not completed, please describe in the "comments" why actual output / deliverable deviated from the proposed.

#	Proposed goal / objective / activity	Target output / deliverable	Completed (Y/N)	Comments	Topical area (s) and research need(s) addressed (as described in the proposal)
1	To improve understanding of the effects of acute SWI on carbon uptake and decomposition in a freshwater marsh, which are two main ecological processes affecting organic matter incorporation and build-up	Evaluating the effects of acute SWI events on carbon fluxes affecting organic matter accretion in wetland patches dominated by two common upper estuary freshwater plants and explore the relationship of those effects with sensible changes in plant phenology (a potential metric of marsh deterioration). We further assessed changes in marsh elevation in relation to those acute SWI events.	Y		**Topic area 1:** Hydrology and hydrodynamics of riverine, and coastal systems. **Research activity #4:** Assessing stressors that cause wetland elevation loss including those that influence the reduction in total wetland vegetation cover or other metrics of marsh deterioration

2. **Peer-reviewed publications.** Please provide .pdf copies of all publications.

Authors	List author names of graduate students/postdocs	Title	Journal	DOI (or other identifier)	Published; submitted; in prep; planned?	Date
Diana Taj	Robert Bordelon	The effects of acute saltwater intrusion on carbon gas fluxes from two common plant species of freshwater wetlands in Louisiana.	Science of the Total Environment	ISSN: 0048-9697 (print), 1879-1026 (web)	In prep.	Planned submission month: March, 2024

3. **Oral presentations and posters.** Please provide .pdf copies.

Presenter	Co-authors	List author names of graduate students/Postdocs	Title	Oral or poster?	Conference or meeting name	Date	Proceedings published? (Y/N)
Diana Taj	Robert Bordelon, Monique Blanchard, Jordan Stoll, Mason Marcantel, Jorge A.Villa	Diana Taj, Robert Bordelon, Monique Blanchard, Jordan Stoll, Mason Marcantel.	The effects of acute saltwater intrusion events on methane and CO_2 Fluxes from *Typha domingensis* and *Panicum hemitomon* wetland vegetation patches	Oral	Society of Wetland Scientists	June 27- 30, 2023	Y
Jorge A. Villa	Diana Taj	Diana Taj	Patch-scale effect of saltwater intrusion on carbon fluxes in coastal areas	Oral	LA-COE RFP 2 all-hands meeting	August 1, 2023	N
Diana Taj	Jorge A. Villa	Diana Taj	Patch-scale effect of saltwater intrusion on carbon fluxes in coastal areas	Poster	LA-COE RFP 2 all-hands meeting	August 1, 2023	N
Diana Taj	Robert Bordelon, Jorge A. Villa	Diana Taj, Robert Bordelon	The effects of a simulated saltwater intrusion event on CO_2 leaf fluxes and porewater concentrations from *Typha domingensis* vegetation patches in a freshwater environment	Poster	American Geophysical Union Fall Meeting	December 12-16, 2022	N
Diana Taj	Jorge A. Villa	Diana Taj	Patch-scale effects of acute saltwater intrusion on carbon fluxes in a simulated coastal freshwater environment	Oral	LA-COE RFP 2 all-hands meeting	August 11, 2022	N

Diana Taj	Robert Bordelon, Jorge A. Villa	Diana Taj, Robert Bordelon	Measuring the Impact of Acute Saltwater Intrusion on Methane and CO_2 Fluxes from *Typha domingensis* Vegetation Patches in a Freshwater Environment	Oral	Joint Aquatic Sciences Meeting	May 14-20, 2022	N
Diana Taj	Robert Bordelon, Jorge A. Villa	Diana Taj, Robert Bordelon	Measuring the effects of acute saltwater intrusion on methane and CO_2 fluxes from freshwater wetland vegetation: An experimental ecosystem-scale approach	Poster	American Geophysical Union Fall Meeting	December 13-17, 2021	N
Diana Taj		Diana Taj	Thesis defense: Measuring the impact of acute saltwater intrusion on methane and CO_2 fluxes from freshwater wetland vegetation patches	Oral	School of Geosciences - University of Louisiana at Lafayette	November 11, 2021	N

4. **List other products or deliverables**. These can include white papers, patent applications, workshops, outreach activities/products. Describe and provide .pdf copies, as applicable.

World Wetland Day celebration at the Lafyette Middle School (Lafayette, LA)

Exposition at the 2022 Mid-Winter Fair Fishing Rodeo (Abbeville, LA)

Participation in LA_COE Co-production of Science Workshop (Baton Rouge, LA)

5. **Data.** Making data publicly accessible in a timely manner is a key goal of the data management policy of RESTORE Act Center of Excellence. All projects must ensure that data and ISO metadata are collected, archived, digitized, and made available using methods that allow current and future investigators to address new questions as they arise. Per the U.S. Department of the Treasury's Office of Gulf Coast Restoration Data Accessibility and Management Best Practices[1] *"Data are generally expected to be made publicly available at the time of publication of a peer- reviewed article relying on the data or two years after the data are collected."* All information products resulting from funded projects must be associated with detailed, machine-readable metadata (ISO format) and shared in a regional or national digital repository or data center (e.g., National Centers for Environmental Information, Gulf of Mexico Research Initiative Information & Data Cooperative, Inter-university Consortium for Political and Social Research, DataOne Dash) for discovery and long-term preservation. Metadata, a brief description of the data, and location of the data (e.g., repository, DOI) must be provided to the LA-COE to enable tracking of all data and information products.

#	**Data Title**	**Data Description**	**Repository or Data Center**	**Date by when it will be publicly available (1 year after final report)**	**DOI link (if already available)**
1	CO_2 and CH_4 fluxes and porewater concentrations, and plant spectral indices during recreated saltwater intrusion on patches dominated by common freshwater plant species of Louisiana.	CO_2 and CH_4 flux, CO_2 and CH_4 porewater concentration, water and soil salinity, water level, vegetation spectral indices (NDVI, PSRI, PSI), soil temperature, biomass	Environmental System Science Data Infrastructure for a Virtual Ecosystem (ESS DIVE)	February, 2023	

[1] https://www.fio.usf.edu/documents/flracep/program-documents/Treasury%20RESTORE%20COE%20data%20management%20best%20practices%20Jan%202018.pdf

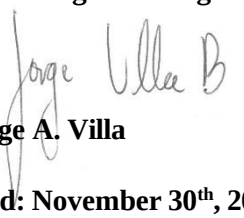
6. **Mentoring and Training.** Please list post-doctoral and graduate and undergraduate student participants (provide .pdf copies of thesis/dissertation).

First Name	Last Name	BS/MS/PhD/Postdoc	# Years involved	Institution	Thesis/Dissertation Title/Research Topic or Tasks	Did the student graduate? (Y/N)	If they graduated, current position/location?
Diana	Taj	MS/PhD	2	UL Lafayette	Measuring the impact of acute saltwater intrusion on methane and CO_2 fluxes from freshwater wetland vegetation patches	Y	The student continued in UL Lafayette and is pursuing a PhD in Earth and Energy Sciences. The dissertation is a continuation of the experimentation that took place in the study.
Robert	Bordelon				NA		NA
Monique	Blanchard	BS	1	UL Lafayette	NA	N	NA
Jordan	Stoll	BS	0.5	UL Lafayette	NA	Y	HDR Engineering. Lafayette, LA.
Mason	Marcantel	BS	0.2	UL Lafayette	NA	Y	Student worker, US Geological Survey – Wetland and Aquatic Research Center. Lafayette, LA.

C. CERTIFICATION

Certification: I certify to the best of my knowledge and belief that this report is correct and complete for performance of activities for the purposes set forth in the award documents.

Principal Investigator: Jorge A. Villa

Signature: 

Name: Jorge A. Villa

Date Signed: November 30th, 2023

Approval: I have evaluated the final report and associated invoice and confirm that the project is finished.

LA-COE Technical Point of Contact:

Signature: Jessica R. Henkel

Name: Jessica Renee Henkel

Date Signed: 12/28/23

Approval: I have reviewed the final report and approve for payment.

LA-COE Director:

Signature: Jessica R. Henkel

Name: Jessica Renee Henkel

Date Signed: 12/28/23